Development and Applications of the Automated Benchtop Dynamic Pressure Generator for High Pressure Bioscience Research

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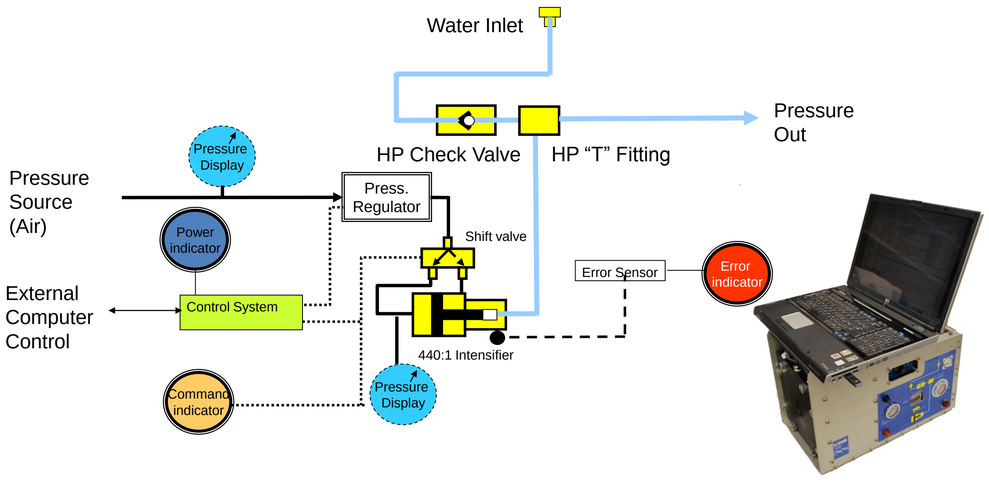
Introduction

Pressure is a significant thermodynamic parameter that is orthogonal to temperature. The work of the 1946 Nobel Prize winner Percy W. Bridgman inspired further research of high pressure thermodynamics in biological systems. However, the limitations of material science and engineering to these days have lead to a prominent lack of commercial laboratory equipment suitable to adequately support high pressure research efforts in life sciences. For decades, high pressure research has been driven by do-it-yourself enthusiasts capable of combining fundamental knowledge of biology, physics and chemistry with the need to practice general plumbing and sophisticated mechanical engineering skills. Majority of the high pressure biochemistry and biophysics work had been done using primitive manual equipment and hours of mandatory physical exercise. The goal of this work is to develop user-friendly dynamically-controlled automated pressure generator suitable for unattended pressure perturbation studies of protein conformation, including enzymology, kinetics of protein folding, aggregation, and self-assembly.

HUB440 Pressure Generator: Flow Diagram



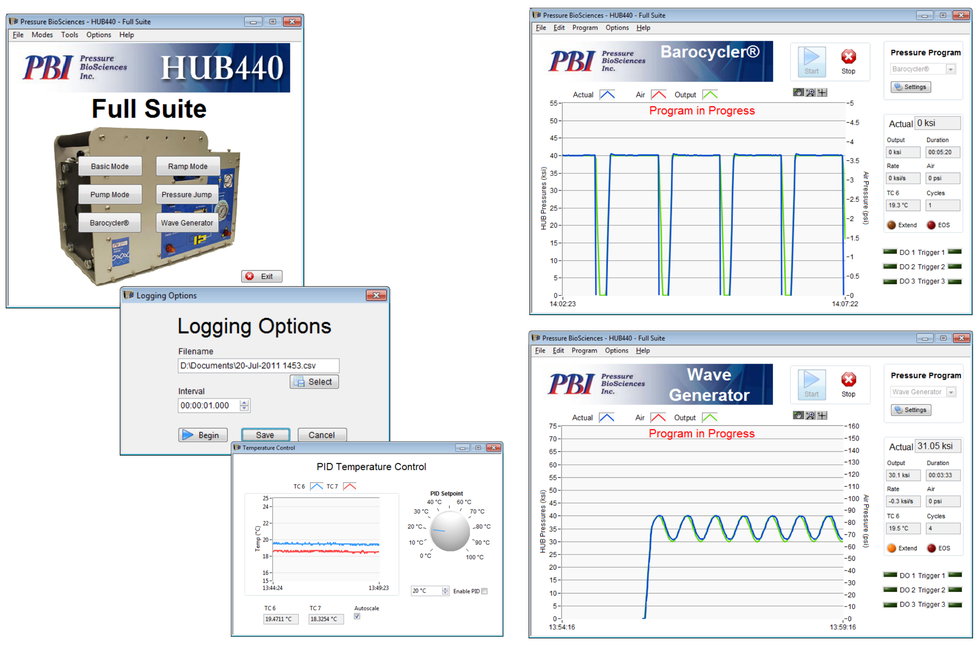
The core of the system is an electronically-controlled air-driven reciprocating pressure intensifier with a fixed ratio of 1:440, single stroke displacement volume of 3.6 mL and a maximum outlet pressure of 4 kBar. The intensifier is equipped with the dynamic high pressure seal system with a minimum friction, resulting in the ability to ramp pressure over time with a resolution of several Bar per second in ascending and descending directions. Water is used as a high pressure media due to its low compressibility. Electronic regulator controls the air pressure and the direction of air flow to the front or the back of the air cylinder, resulting in a precise extension or retraction of the intensifier piston. Pressure is controlled via manual control panel on the front of the instrument or remotely by the USB-powered Data Acquisition and Control interface. Built-in high pressure transducer permits real-time pressure feedback. The system is powered by a 24 VDC power supply. High pressure check valves prevent the back flow of water and enable rapid refill of the intensifier with water for multi-stroke operation. Open frame packaging of the instrument provides easy access to all serviceable components and enables rapid customization. High pressure outlet is equipped with the standard high pressure type "T" fitting compatible with a wide variety of industry-standard high pressure equipment.

Barocycler ™HUB440 Software Control Interface

Computer control via optional USB-powered Data Acquisition and Control interface offers flexible programming of pressure values. Intuitive software also offers multiple channels of data acquisition, including logging of pressure, temperature and outputs from additional sensors. Digital trigger inputs and outputs offer several options for integration with external equipment such as optical and magnetic resonance spectrometers and HPLC components.

Hardware: National Instruments USB-6211 DAQ Card, Electronically controlled air valves, Microsoft Windows XP/Vista/7 computer with available USB 2.0 port.

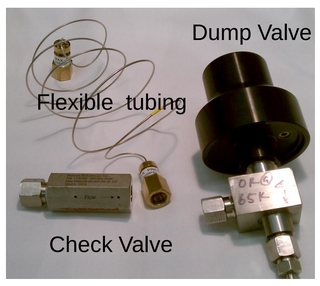
Software: 32-bit and 64-bit software versions are available with standard functionality: Basic pressure control, large vessel pumping, wave generator , Barocycler and Pressure Jump. Custom software development is possible using LabVIEW 2010.



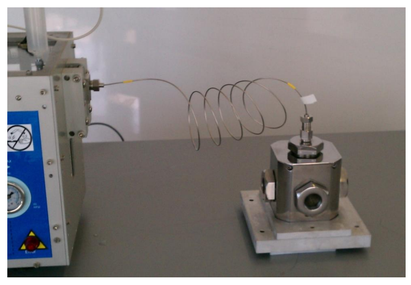
Specialized Peripheral High Pressure Components

Chamber, 30 mL

Chamber, 10 mL

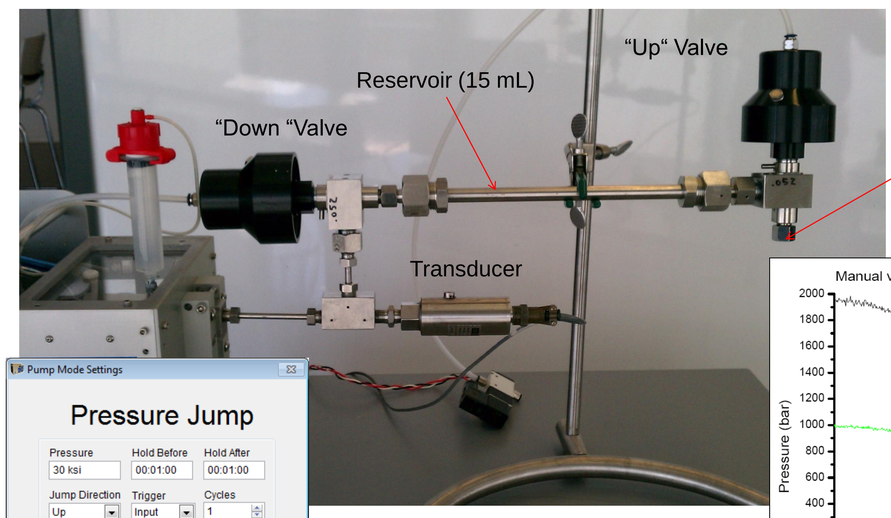


- Ultra-fast pressure-jump valves
- High pressure chambers
- High pressure check valves
- High pressure tubing adapters
- Caps, connectors and tees

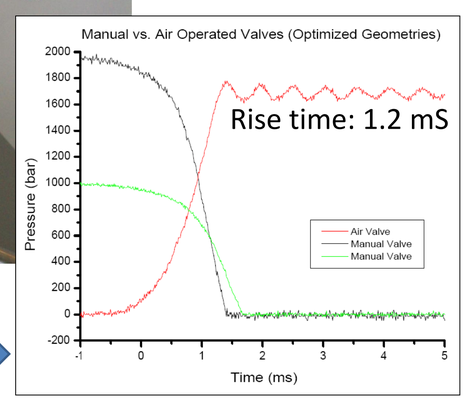


Standard HP fittings allow connection to the ISS Instruments high pressure cell (pictured above) which enables UV-Vis absorbance and fluorescence measurements up to 4 kBar.

Pressure Jump Setup for Rapid Pressure Perturbation Kinetics Experiments



Pressure Jump Data recorded with the high speed piezoelectric transducer at Dr. Hubbell's laboratory (UCLA)

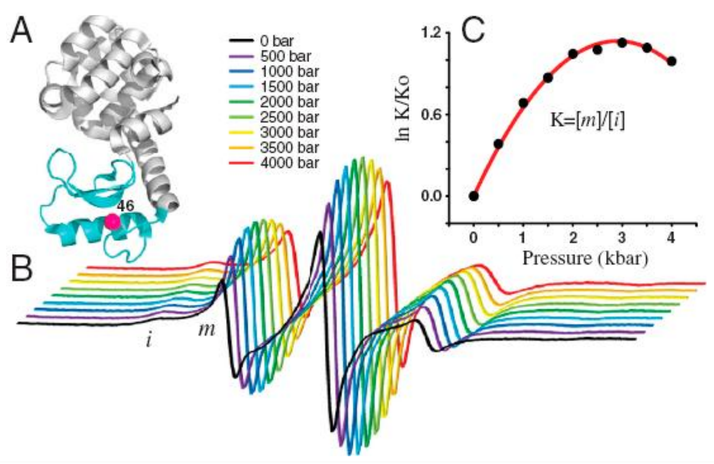


High pressure EPR using Site-Directed Spin Labeling (SDSL)

HUB440 is used to pressurize fused silica capillary EPR cell on a Bruker EleXsys 580 EPR spectrometer fitted with the high sensitivity cavity. SDSL-EPR allows direct determination of pressure-dependent equilibrium constants for protein conformational equilibria.

McCoy J., Hubbell W. L., 2011 [1]

$$\ln\frac{K(P)}{K(0)} = -\frac{\Delta\bar{V}^o}{RT}(P) + \frac{\Delta\bar{\beta}_T}{2RT}(P)^2$$



(A) Ribbon diagram of T4 lysozyme mutant T4L46R1 (PDB ID code 3LZM) showing the location of residue L46.

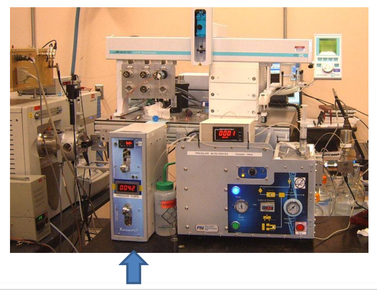
(B) The pressure dependence of the EPR spectra normalized to the same number of spins.

(C) The equilibrium constant determined from fits to the spectra is plotted as indicated *vs.* pressure (dots); the solid line is a fit to theoretical equation shown above.

Proteomics: On-line Tryptic Digestion in a Flow-through Pressure Chamber

The HUB440 pressure generator has been used to pressurize the 200µL flow-through Pressure Transfer Cell (PTC) for on-line pressure-enhanced tryptic digestion of protein samples directly prior to UHPLC separation and analysis by FT-ICR mass spectrometer. The PTC isolates sample/HPLC Mobile phase flow from any contact with the hydrostatic pressure media (water).

Hyung *et al*. 2011 [2]



Conclusions

We have developed an automated high pressure generator capable of unattended dynamic pressure control up to 4 Kbar. The flexible software control interface has also been designed. This system enables rapid integration with a wide variety of analytical instruments such as spectroscopy and chromatography equipment.

Possible applications for the new system relevant to protein research include high pressure perturbation of protein conformation, thermodynamics of protein folding, control of enzyme and chemical reaction kinetics, analysis of protein aggregation and amyloid self-assembly, optimization of protein crystallization, studies of protein-lipid interactions and phase transitions of lipid bilayer, compressibility measurements, deep sea biology, vaccine development and pathogen inactivation.

The new instrument is expected to lower the barrier for adoption of high pressure research in many life sciences laboratories leading to the broader awareness of the biophysics and biochemistry research community to the benefits of high pressure bioscience.

References

[1] J. McCoy, W. L. Hubbell. High-pressure EPR reveals conformational equilibria and volumetric properties of spin-labeled proteins. (2011) Proc Natl Acad Sci U S A. 108(4):1331-6.

[2] S.-K. Hyung *et al*. Development of a 20 kpsi Enzymatic Digester for High Throughput Proteomic Analysis and Its Application to Membrane Proteomics. Poster at the 59th ASMS Conference, Denver, CO, June 5-9, 2011 http://www.pressurebiosciences.com/downloads/publications/2011-06/Hyung_ASMS_2011.pdf